Experimental Acute Lead Encephalopathy in the Juvenile Rhesus Monkey*

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Lead subacetate (0.5g) and 1000 units of vitamin D were given three times a week to four newly-weaned rhesus monkeys. In addition, two animals received only the vitamin D. The poisoned animals had an increase in the urinary excretion of δ-amino-levulinic acid, an elevated content of lead in the blood, and a fall in hemoglobin concentration. Between 6 and 18 weeks the animals suddenly developed ataxia, nystagmus, generalized weakness, and convulsions. At this time the animals were killed by perfusion of fixative and the brain prepared for light and electron microscopic studies. Definite morphological evidence of disease was confined to the central nervous system, except for one animal which showed the characteristic renal inclusions of lead poisoning. All animals showed PAS-positive globules associated with blood vessels and an exudative edema involving the white matter of the cerebral hemispheres and cerebellum. Ultra-structurally, this appeared as a granular precipitate within an expanded extracellular space. Alterations of nerve fibers were not seen in the white matter but axonal swelling was observed in the cerebral cortex. The perikaryon and neuropil appeared normal. The control animals showed no significant cerebral changes.

Introduction

In this paper we report the successful production of acute lead encephalopathy in four juvenile rhesus monkeys by the combined administration of oral lead and vitamin D. This experimental model resembles the human disease more closely than encephalopathy produced by lead in the rat (1).

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Methods

Newly weaned rhesus monkeys, 5–6 months of age, were obtained from the Perinatal Biology Laboratory of our institution. All animals were born on the premises and were fed the normal monkey diet after weaning. The animals were given 0.5 g of lead subacetate and 1000 units of vitamin D dissolved in corn oil by gastric gavage. In all but one case this was given every other

day three times a week. The vitamin D was given to enhance the alimentary absorption of lead (2). Four animals were given lead and vitamin D. Two animals, given only the vitamin, served as controls. Periodically, the animals were tranquilized with phency-clidine hydrochloride (Sernylan, Bio-ceutic Laboratories St. Joseph Missouri) and weighed. At this time a complete blood count was done on capillary blood by utilizing a Coulter Model S instrument. The 24-hr urine samples were obtained by placing the animal in a metabolism cage and collecting the urine over solid carbon dioxide. The δ-aminolevulinic acid (ALA) was determin-ed by the method of Davis and Andelman (3). Blood for lead analysis was obtained by femoral vein puncture. The blood was dry-ashed at 500°C in a platinum crucible and resuspended at pH 2.5 using 10% tri-chloracetic acid and sodium hydroxide. This was followed by chelation with sodium die-thyldithiocarbamate and extraction with methyl isobutyl ketone. The amount of lead was determined by atomic absorption (Per-kin-Elmer, Model 214).

When definite neurologic abnormalities developed, the animals were anesthetized with pentobarbital and killed by perfusion of fixative through the abdominal aorta with the lower portion ligated. The brain was prepared for light and electron microscopic studies as previously described (1). Tissues obtained from the viscera, muscle, and peri-pheral nerve were embedded in paraffin and stained by routine methods. Control animals were killed in a similar manner after 8 weeks of exposure to vitamin D. For electron microscopy, samples of grey and white mat-ter were taken from the precentral gyrus, post-fixed in osmium tetroxide, embedded in Epon, and studied with an RCA electron microscope (Model EMU 3–G). One femur was removed and the lead content determined as described above following removal of the bone marrow.

Results

Physiologic Observations

Controls: Hematologic data are sum-marized in Table 1. These figures are based on 14 blood counts obtained from six juvenile rhesus monkeys. They include values for the four animals prior to administration of lead. The hemoglobin, urinary ALA, and weight are recorded in Figure 1 for control female monkey, Beta. The maximal urinary ALA content in this animal was 690 μg/

Table 1. Hematologic data.

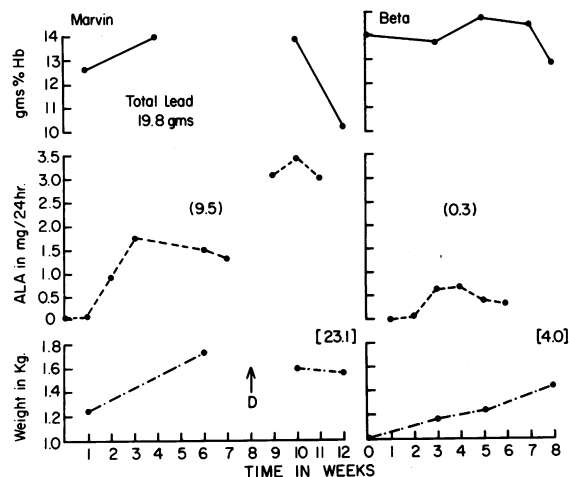|	Mean x	Standard error Sx
Hemoglobin (Hgb). g/100 ml	13.9	0.3
Red blood cells (RBC) 10^6/mm^3	5.94	0.2
Hematocrit (Hct), %	43.7	0.7
Mean corpuscular volume (MCV), μ^3	73.7	1.4
Mean corpuscular hemo-globin (MCH), pg	23.6	0.5
Mean corpuscular hemo-globin concentration (MCHC), %	32.1	0.2
White blood cells (WBC) 10^3/mm^3	8.5	0.8
Neutrophils, %	45.9	4.8

FIGURE 1. Hemoglobin, urinary ALA, and weight for Marvin and Beta. Blood lead level in micrograms per gram weight shown in parenthesis. Time indi-cated by level on abscissa. Lead content of bone in micrograms per gram weight shown in square brackets. D denotes beginning of vitamin D.

day. The animal showed an average weight gain of 56 g/week in this time period. The second control, a male (data not shown in Figure 1), had a hemoglobin of 14.9 g in the third week after beginning vitamin D and 15.7 g/100 ml in the seventh week. Urinary ALA content varied from 250 to 350 μg/24 hr. No lead was demonstrated in the terminal blood sample. The average weekly weight gain was 53 g/week. The bone showed 8 μg of lead/g of bone.

Marvin: This was the first animal in the series. For the first seven weeks he was given 0.5 of lead subacetate three to five times a week. This produced a blood lead level of 950 μg/100 ml without anemia or weight loss (Fig. 1). The urinary ALA rose to a maximum of 1.75 mg/24 hr. Following the addition of vitamin D the urinary ALA content rose to 3.40 mg/24 hr. Four weeks later, the animal suddenly developed symptoms of ataxia, nystagmus, and opisthotonos. The x-rays of the long bones obtained at this time were normal. There was a terminal anemia with the MCV reduced to 60 μ^3 and the MCH to 20.3 μg. The MCHC was 33.0%. The total lead given to this animal was 19.8 g.

Ivan: In this and subsequent animals the standard dose of vitamin D, and lead was used throughout the experimental period. The total lead exposure equals the number of weeks times 1.5. This animal showed a rather marked elevation of the urinary ALA to 4.65 mg/24 hr by the fourth week (Fig. 2). This was accompanied by a failure to gain weight and a progressive fall in hemoglobin, resulting in an enemia with a MCV of 61 μ^3, a MCH of 20.8 pg, and a MCHC of 34.2%. Normoblasts and basophilic stippling appeared during the fourth week. At the beginning of the seventh week the animal developed lethargy, anorrhexia and muscular weakness. On the next day convulsions appeared, and the animal was killed. Just prior to the onset of symptoms, the animal developed a leukocytosis of 32,500 mm³ with 62% neutrophils.
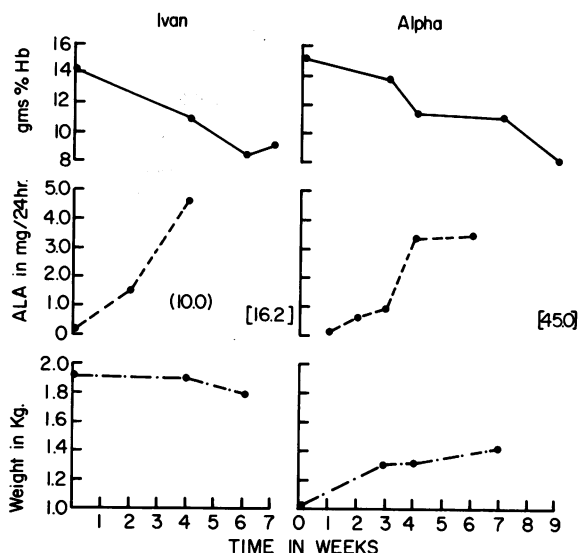
Alpha: This animal showed a course

FIGURE 2. Hemoglobin, urinary ALA, and weight for Ivan and Alpha. Symbols as in Fig. 1.

similar to the one just described. After the third week the growth rate was reduced to 32 g/week. Terminally, there was an anemia with a MCV of 62 μ^3, a MCH of 20.5 pg, and a MCHC of 33.3%. He also developed a leukocytosis of 16,300/mm³ with 62% neutrophils. At the end of the eighth week the animal became anorrhexic. Although he still could take a piece of apple, he seemed unable to grasp food with his right hand. Later in the day he appeared to be improved. The following day he was ataxic and salivating. He was unable to sit up but showed little loss of motor strength. There was little or no response to pain. Just prior to killing the animal developed a right-sided convulsion.

Minne: This female monkey showed a more protracted course (Fig. 3). Although the urinary ALA excretion was increased, this was not sustained above a level of 3 mg/day. The hemoglobin dropped slightly. Although there was not a terminal anemia, the RBC constants were diminished (MCV, 63 μ^3; MCH, 19.1 pg; MCHC, 30.4%). The growth rate for the first 3 months was normal (52 g/week). Between the 12th and 15th week it fell to 20 g/week. At the end of the 16th week the animal appeared
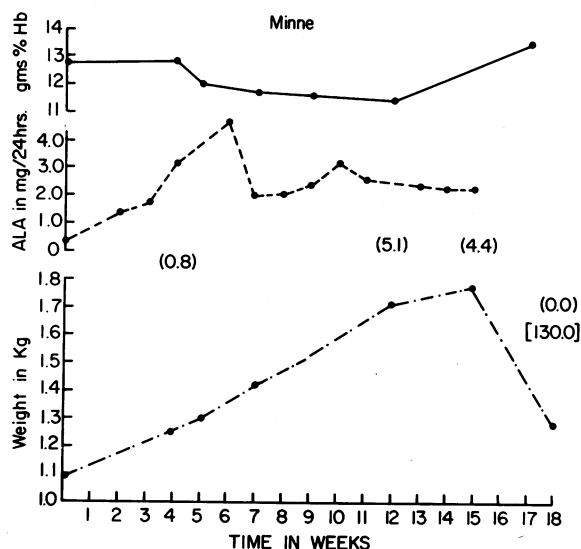
FIGURE 3. Hemoglobin, urinary ALA, and weight for Minne. Symbols as in Fig. 1.

weak, but improved. Five days later generalized weakness, greater on the right, again appeared and the animal was killed. It is of interest that, although the blood lead level was found to be elevated throughout the course, none was found in the terminal sample. Terminally, there was a peripheral blood leukocytosis of 58,100/mm³ with 69% neutrophils.

Careful funduscopic examinations were made of all animals throughout the experimental period and terminally. There was no papilledema.

Morphologic Changes

The histologic appearances of the viscera, voluntary muscle, and peripheral nerves of all animals were within normal limits, with the exception of the kidneys of Minne. These showed the characteristic intranuclear inclusions of lead poisoning.

The brains of control monkeys showed no changes by light or electron microscopy.

When compared to the controls, all of the lead-poisoned monkeys showed gross evidence of herniation of the cerebellar tonsils (Fig. 4).

Microscopically, perivascular glial nodules

were prominent in the spinal cord and brain stem (Fig. 5). These perivascular cells were not impregnated in the gold chloride technique but stained well with the silver methods for microglia. Perivascular periodic acid-Schiff (PAS)-positive globules were found in grey and white matter of the brain stem, cerebellum and cerebrum. They were most prominent in the cerebellar grey and the cerebral white matter. The globules stained red with the Phloxin Fast Green gallocyanin (PFG) technique (Fig. 6). They were acid-fast and were negative for iron by the Prussian Blue reaction and for inorganic anions by the von Kossa technique. With the PFG a thin rim of glial cytoplasm could often be seen around the structures. This cytoplasmic rim was also evident in the gold chloride technique. By electron microscopy, these inclusions were the same as those described previously in the rat and man (1). They were predominantly within the processes of astrocytes. Unlike the rat, but like the human, these inclusions were not confined to this location. They were also seen within endothelial and perithelial cytoplasm.

The histologic basis for the grossly observed swelling was the accumulation of a PAS-positive edema fluid between the nerve fibers of the cerebellar and cerebral white matter (Fig. 7). Between such areas the fibers were often separated but the interstices appeared empty. The edema fluid stained green with the PFG technique. It was also found in the subpial zone and around Purkinje cells. By electron microscopy, the edema fluid appeared as a granular precipitate separating apparently normal nerve fibers (Fig. 8). The astrocytes in the white matter in both locations were quite prominent with both the Holzer and gold chloride techniques. Their cytoplasm was often PAS-positive and stained green with the PFG technique (Fig. 6). As with the rat, macrophages with PAS-positive cytoplasm were prominent.

The cerebral cortex of all the poisoned animals showed focal vacuolated areas (Fig. 9). With the Luxol Fast Blue stain these vacuoles were often found to be lined by

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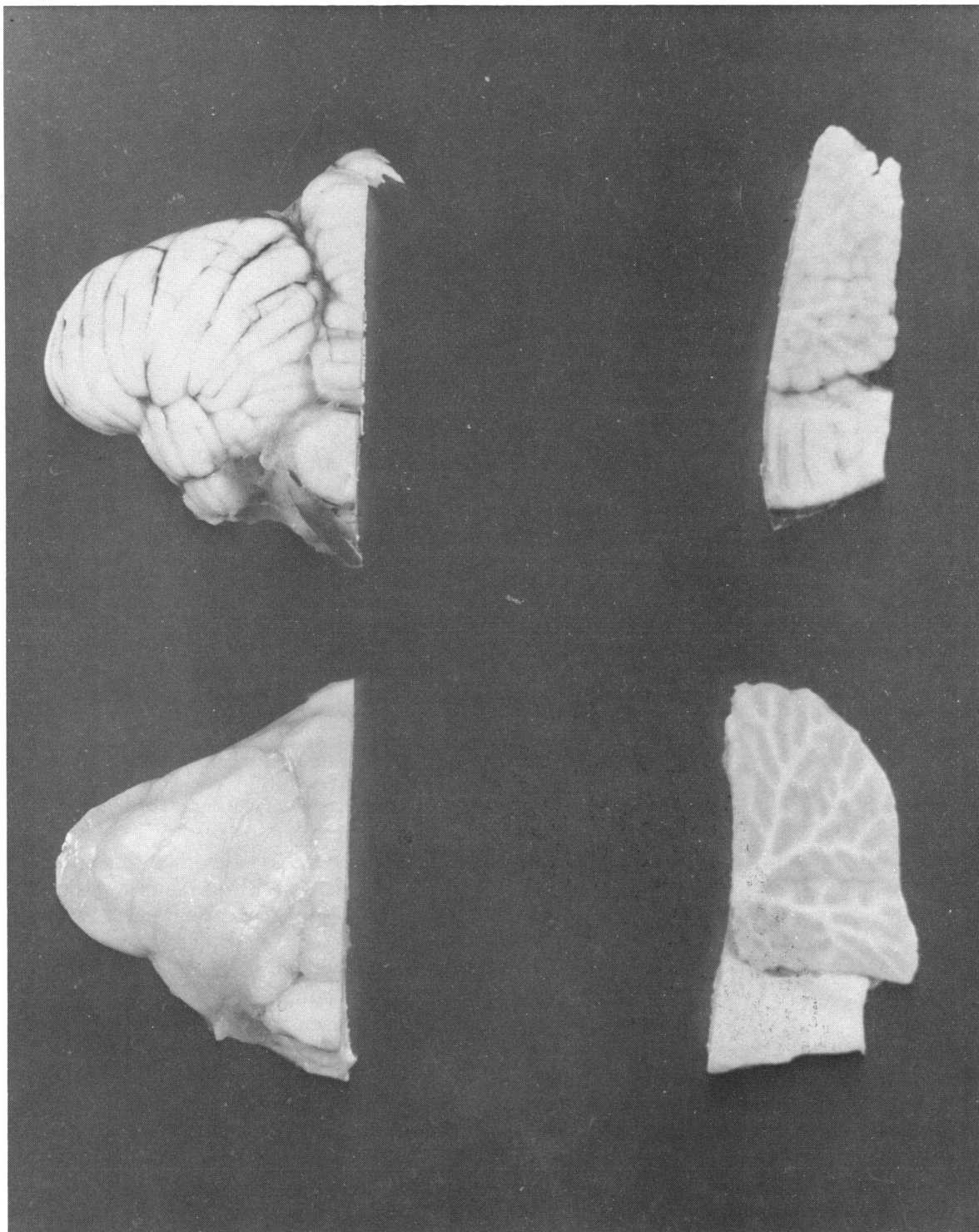
FIGURE 4. Photographs of (top) normal monkey brain fixed by immersion in formalin and (bottom) Marvin, brain fixed by glutaraldehyde perfusion. The cerebellar tonsils are herniated.
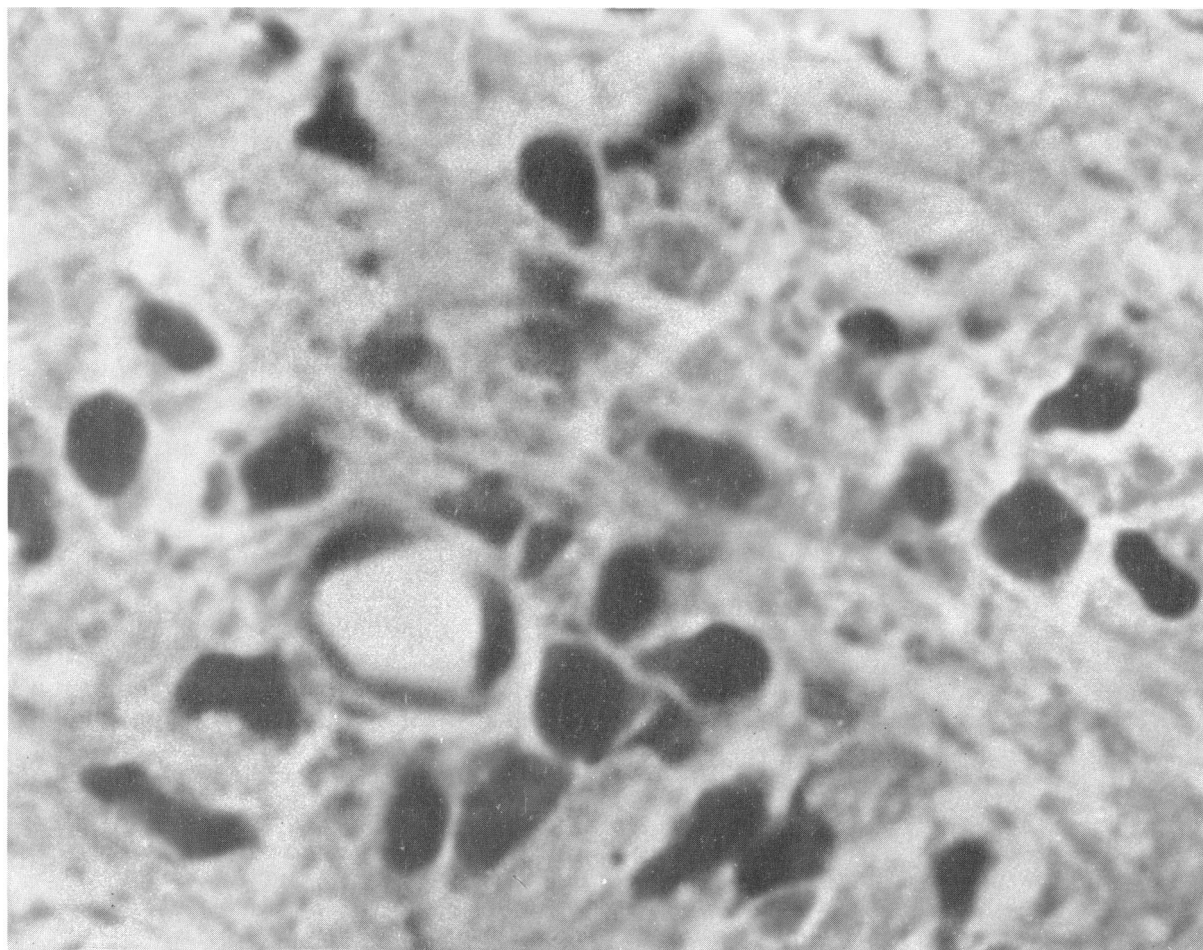
FIGURE 5. Perivascular glial nodule from spinal cord of Marvin. Hematoxylin-eosin stain. ×700.

a thin sheet of myelin, suggesting that they were swollen axis cylinders. By electron microscopy this was, indeed, found to be the case (Fig. 10).

Discussion

The experimental disease described in this report resembles human acute encephalopathy much more closely than that previously reported in the rat (1). As in the rat, edematous changes are seen in the cerebellar and subcortical white matter, but in the monkey the basal ganglia are not involved. The cerebellar petechial hemorrhages which so dominate the morphological changes in the rat are essentially absent in

the monkey. The involved white matter in the monkey shows a diffuse astrocytosis, and glial nodules are also prominent. Furthermore, in the monkey the associated nutritional changes reflected in severe weight loss and fur changes are not seen.

The perivascular PAS-positive globules were not, as they are in the rat, confined to the cytoplasm of astrocytic processes. As was discussed in a previous paper (1), we consider the perivascular PAS-positive globules and the exudative edema to be characteristic of acute lead encephalopathy. We have recently had the opportunity to examine sections from the brain of a baboon who was exposed to lead and died spontaneously (4). This animal had both lesions
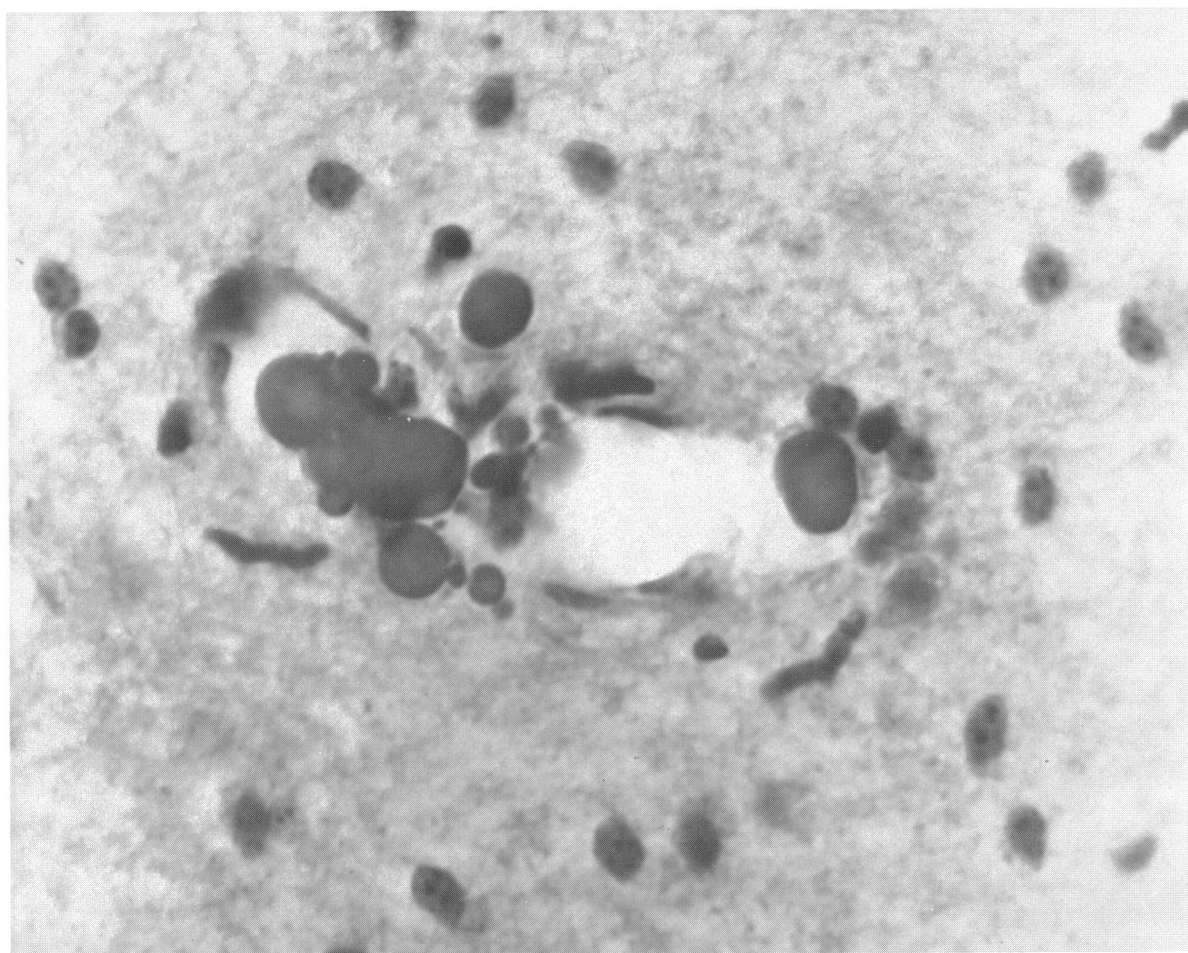
FIGURE 6. Subcortical white matter of Marvin. Perivascular nodules of varying size are seen. The astrocytes in the adjacent white matter are also abnormally prominent. PFG stain. ×500.

in the cerebellum. The perivascular globules were particularly conspicuous.

The necrobiotic neuronal changes observed in about half of our human cases (1) were absent in the monkey and the rat. A lesion not seen in the rat was the swelling of myelinated axons without accompanying alteration of the myelin sheath. Although a similar change has been described in human biopsies studied by electron microscopy (5), we have not observed the light microscopic equivalent of this lesion in our human material. A similar lesion has been described in experimental cyanide poisoning (6). Cyanide

is known to affect cytochrome oxidase (cytochrome a + a^3). This substance is also diminished in the mitochondria of the kidney in lead-poisoned rats (7). The axonal swelling may therefore reflect an effect of lead on cytochrome oxidase.

It has been reported that loss of weight is the most striking feature of lead poisoning in baboons but no consistent fall in hemoglobin was observed (8). All of our poisoned animals showed disturbances in body weight 2–3 weeks prior to the onset of acute encephalopathy. This would appear to be the best prognostic sign of impending disease. Unlike
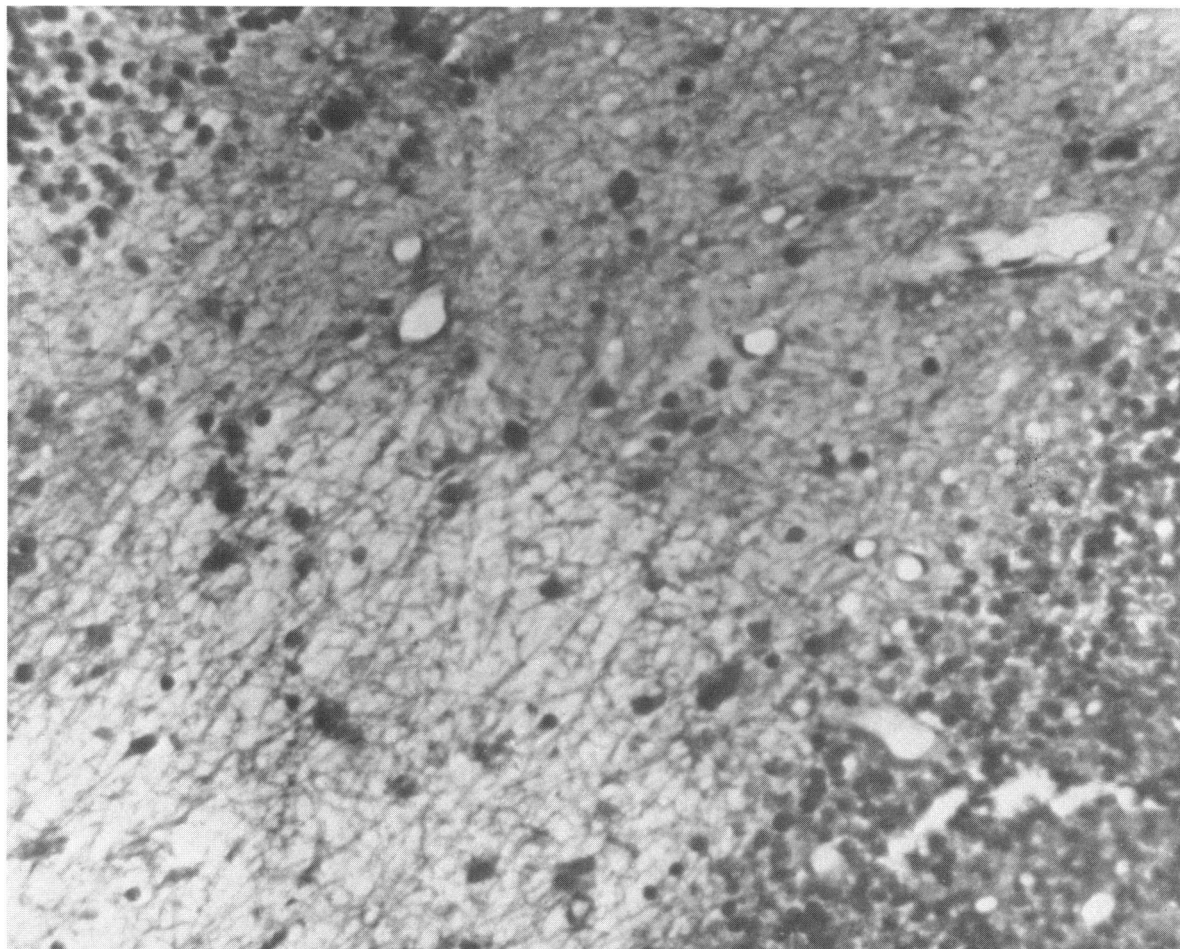
FIGURE 7. Cerebellar white matter of Marvin. The myelinated fibers are separated. In the upper portion of the field the interstices contain PAS-positive edema fluid. Phagocytic cells with PAS-positive cytoplasm are also prominent. Luxol Fast Blue–PAS–hematoxylin stain. ×180.

the baboon, the hemoglobin concentration fell at some time during the course of the poisoning in all our animals and terminally all showed microcytic hypochromic erythrocytes. Three of the four animals had a terminal leukocytosis and in the fourth a leukocyte count was not done. These hematologic data also may have prognostic significance. It is difficult to explain why one monkey, Minne, the only female, was relatively resistant to the effects of lead. This may be related to the fact that this animal did not develop a sustained elevation of the urinary ALA to above 3.0 mg/day.

The findings in the two "control" animals, which received only vitamin D, indicate that there may have been increased absorption of lead normally present in the environment occurring as a result of the administration of the vitamin. Because of a lack of a true control group of animals not receiving vitamin D, we cannot be certain of this. The urinary ALA figures for Charley are within the range of the initial values in the lead-poisoned monkeys but the higher values seen in Beta may not be. This problem will require further study.
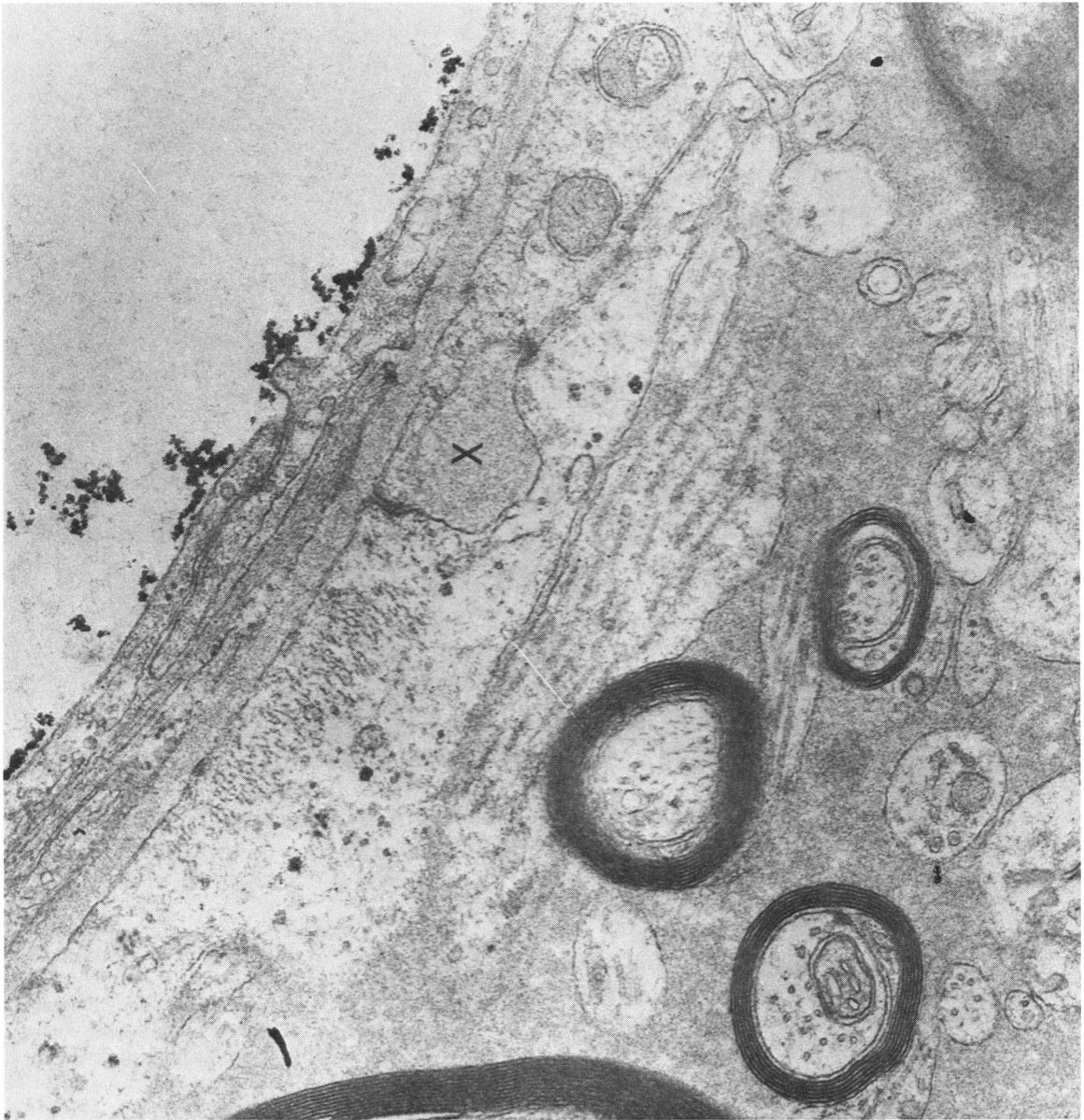
FIGURE 8. Subcortical white matter of Alpha. Edema fluid appears as a granular precipitate in the expanded extracellular space adjacent to an apparently normal blood vessel. This fluid is also seen in a space between two perivascular astrocytic processes (X). × 46,390.
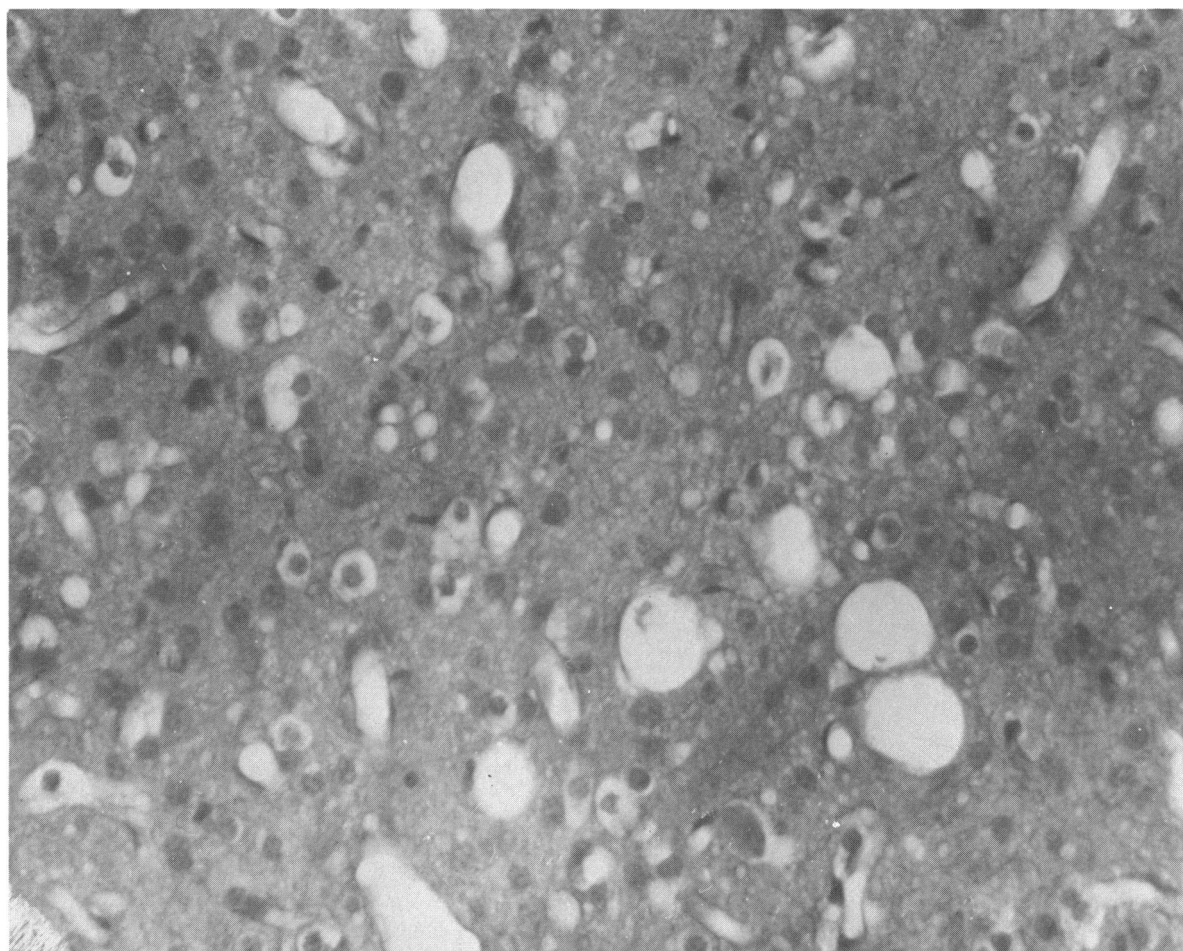
FIGURE 9. Motor cortex of Alpha. Vascuolated areas are prominent. Luxol Fast Blue–PAS–hematoxylin stain. ×200.

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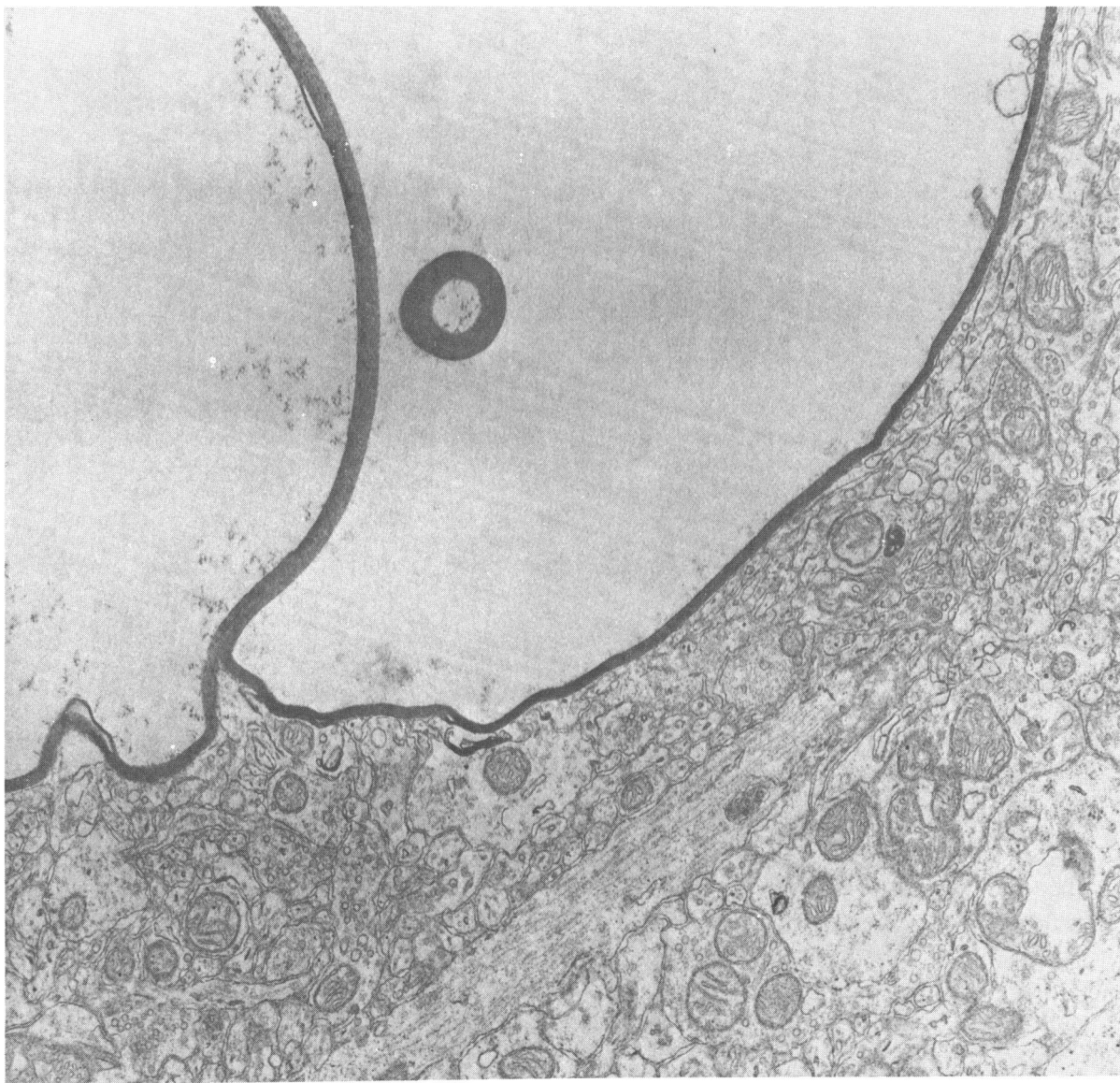
FIGURE 10. Motor cortex of Alpha. Two swollen axons are seen on the left. One of these contains a myelin figure. There are no definite alterations of the myelin sheath and the adjacent neuropil appears to be normal. ×27,870.